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Enhanced Th1 Response to Staphylococcus aureus Infection in Human Lactoferrin-Transgenic Mice

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Lactoferrin (Lf) is an iron-binding protein of external secretions and neutrophil secondary granules with antimicrobial and immunomodulatory activities. To further define these properties of Lf, we have investigated the response to Staphylococcus aureus infection in transgenic mice carrying a functional human Lf gene. The transgenic mice cleared bacteria significantly better than congenic littermates, associated with a trend to reduced incidence of arthritis, septicemia, and mortality. We identified two pathways by which S. aureus clearance was enhanced. First, human Lf directly inhibited the growth of S. aureus in vitro. Second, S. aureus-infected transgenic mice exhibited enhanced Th1 immune polarization. Thus, spleen cells from infected transgenic mice produced higher levels of TNF-α and IFN-γ and less IL-5 and IL-10 upon stimulation ex vivo with the exotoxin toxic shock syndrome toxin-1 compared with congenic controls. To confirm that these effects of Lf transgene expression could occur in the absence of live bacterial infection, we also showed that Lf-transgenic DBA/1 mice exhibited enhanced severity of collagen-induced arthritis, an established model of Th1-induced articular inflammation. Higher levels of stainable iron in the spleens of transgenic mice correlated with human Lf distribution, but all other parameters of iron metabolism did not differ between transgenic mice and wild-type littermates. These results demonstrate that human Lf can mediate both antimicrobial and immunomodulatory activities with downstream effects on the outcome of immune pathology in infectious and inflammatory disease. The Journal of Immunology, 2002, 168: 3950–3957.

Lf is a iron-binding protein closely related in structure to the plasma iron transport protein transferrin. It is found mainly in external secretions, such as breast milk, and in the secondary granules of neutrophils. Several physiological functions have been attributed to Lf, including broad spectrum antimicrobial activity and anti-inflammatory and immunoregulatory activities (1), mediated either through iron sequestration (2) or by direct interaction with the cell surface (3), which can cause increased bacterial cell membrane permeability (4). Lf can bind iron at sites of inflammation and thus prevent free iron from being available to catalyze harmful free radical reactions (5). It can also affect T cell proliferative responses (6, 7), and various other immunomodulatory roles have been proposed (Refs. 8 and 9 and reviewed in Ref. 10). However, very little is known about how Lf modulates immune responses.

We have previously investigated the role of Lf in Staphylococcus aureus infection, using a murine septic arthritis model (11). The appearance of multiple drug resistance has reawakened interest in natural host defense mechanisms against this organism. In particular, S. aureus is the most frequent etiological agent implicated in septic arthritis, which is characterized by severe articular destruction often complicated by septicemia with associated morbidity and mortality (12, 13). The disease can be reproduced in a murine model in which i.v. injection of an exotoxin-producing strain of S. aureus leads to development of severe septic arthritis, morbidity, and septicemia (14). Using this model, we have shown that local (periarticular) injection of human Lf (hLf) can reduce the severity of both joint inflammation (11) and collagen-induced arthritis (CIA), a model of human autoimmune arthritis. It was hypothesized that Lf acts locally by scavenging potentially damaging free iron from the inflamed joint. However, Lf could also exert a more general effect on immune responses in these models, but this was not investigated.

To address the possible role of hLf as an immunomodulatory molecule, we have examined the response to S. aureus of mice carrying a functional hLf gene. These mice express Lf at high levels in milk, but also in other tissues at lower levels (P. van Berkel and R. W. de Winter, unpublished observations). Thus, hLf is present systemically and constitutively in these mice, which means that it could influence the type of immune response that occurs in the initial stages of infection. We demonstrate here that the hLf-transgenic mice showed enhanced production of IFN-γ and TNF-α and reduced IL-5 and IL-10 production by spleen cells in vitro, indicating enhanced Th1 polarization of the cell-mediated immune response in the hLf-transgenic mice. This was associated with increased ability to clear the bacteria and a trend to reduced development of...
septicemia, arthritis, and mortality compared with their wild-type littermates. In contrast, the development of CIA, in which Th1 responses exacerbate the early stages of disease (15), was more severe in the transgenic animals. Together, these results indicate that Lf promotes a Th1 response, and that its therapeutic potential in infectious and inflammatory conditions depends upon the nature of the immune polarization in disease.

Materials and Methods

Animals

hLf-transgenic mice (line 2261) and wild-type littermates (strain B6CBA) generated as previously described (16, 17) were used to establish a colony at Glasgow University (Glasgow, U.K.). For the CIA model, the transgenic mice were backcrossed on to the DBA/1 background and used after six backcrosses. In all cases expression of the transgene was monitored by tail-typing, and nontransgenic littermates were used as controls in the experiments described below.

Infection with S. aureus

Infection with S. aureus was conducted as described previously (11). Briefly, adult hLf-transgenic mice and age- and sex-matched nontransgenic littermates were injected i.v. with S. aureus strain LS-1 (10^7 CFU, except where otherwise indicated), a strain previously shown to produce arthritis as well as septicemia and morbidity (14). Arthritis and paw thickness were monitored daily for 2 wk using an arthritic score on a scale of 0–3 as defined previously (11), and evidence of sepsis (e.g., hunched appearance, raised fur, reduced spontaneous movement, lethargy) was noted (18). Mice (n = 3 per group) were killed before or 1, 2, 3, or 7 days after i.p. staphylococcal injection, and tissues were removed for histological, bacterial, biochemical, and immunological examination. The presence of bacteria in infected arthritic paws and the left kidney was determined as described previously (11). Preliminary experiments showed that these were the only tissues that contained an appreciable number of S. aureus.

Collagen-induced arthritis

Arthritis was induced as described previously (11). Briefly, hLf-transgenic mice on a DBA/1 background and age- and sex-matched nontransgenic littermates were injected intradermally with 0.2 mg bovine collagen in CFA, followed 3 wk later by another 0.2 mg i.p. injection (without CFA). Mice were monitored for arthritis as described above.

In vitro stimulation of spleen mononuclear cells for proliferation and cytokine production

Spleen single-cell suspensions, obtained by gently mincing spleens, followed by filtration through a cell strainer (BD Biosciences, Franklin Lakes, NJ), were cultured in U-bottom 96-well culture plates at 2 × 10^5 cells/well for up to 72 h at 37°C in RPMI 1640 (Life Technologies, Paisley, U.K.) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (all from Life Technologies). Cells were stimulated with the staphylococcal exotoxins toxic shock syndrome toxin (TSST)-1 (10–100 ng/ml) and staphylococcal enterotoxin A (SEA; 100 ng/ml; both from Toxin Technology, Sarasota, FL) or heat-killed S. aureus (10^6 CFU/ml). Preliminary experiments were performed to establish the optimal concentrations of these stimulatory factors. Proliferation was determined by addition of 1 µCi [3H]thymidine (Amersham Life Sciences, Little Chalfont, U.K.) to triplicate wells 6 h before harvest, and cell 3H activity was counted in a beta counter (1205 Betaplate; Pharmacia Biotech, Uppsala, Sweden).

Determination of growth of S. aureus in vitro

A chemically defined medium (CDM) for growth of S. aureus (19) was rendered iron free by treating with Chelex-100 resin. A stock culture of S. aureus LS-1 was subjected to three passages on nutrient agar plates supplemented with 800 μM ethylenediaminedihydroxyacetic acid to reduce levels of stored iron, and a colony was then seeded into 1 ml CDM and cultivated overnight at 37°C. This culture was used to inoculate 25 ml CDM, with appropriate additions, at an initial OD of 0.1 at 470 nm, and growth was assessed spectroscopically. Iron was added as required in the form of iron-nitrilotriacetate, prepared by carefully mixing freshly prepared ferric chloride in 0.001 M HCl with a 4-fold molar excess of sodium nitrilotriacetate.

Immunohistochemistry

Parafﬁn sections were defrosted and stained with a 4-fold molar excess of sodium ferricyanide and 2% hydrochloric acid for 30 min. Sections were washed several times in distilled water and counterstained lightly with 1% neutral red for 10 s. Finally, sections were washed in water, dehydrated, cleared, and mounted in DPX (Raymond A. Lamb, Eastbourne, U.K.).

Immunohistochemical staining. Frozen sections were incubated with Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA) was added for 30 min. Peroxidase activity was blocked by incubating the sections for 30 min in 0.3% H2O2 in methanol. Sections were incubated with Vectastain Elite ABC reagent (Vector Laboratories), washed in PBS, and incubated in peroxidase substrate solution until the desired stain intensity developed. Finally, sections were rinsed in tap water, counterstained with hematoxylin, cleared, and mounted in DPX.

Statistical analysis

Statistical analysis was performed using Student’s t, Mann-Whitney, or χ2 test as appropriate. Values of p < 0.05 were considered significant.

Results

Immunoregulatory effects of hLf overexpression in mice infected with S. aureus

Plasma cytokines. Increased levels of IFN-γ were present in plasma 1 day after infection, with lower levels detected from day 2, but there was no significant difference between the groups (Fig. 1). Significant elevation of plasma TNF-α was also observed in both hLf-transgenic and wild-type mice on day 3 after infection, but again there was no significant difference between the two groups (data not shown). No IL-4 or IL-5 expression was detected in the plasma of any infected group.

Immunohistochemistry

Perls Prussian blue reaction (for ferric iron). Paraffin sections were transferred to a fresh solution of equal parts of 2% aqueous potassium ferrocyanide and 2% hydrochloric acid for 30 min. Sections were washed several times in distilled water and counterstained lightly with 1% neutral red for 10 s. Finally, sections were washed in water, dehydrated, cleared, and mounted in DPX (Raymond A. Lamb, Eastbourne, U.K.).

Immunohistochemical staining. Frozen sections were defrosted and transferred to 10 mM sodium phosphate (pH 7.6). Sections were incubated for 30 min with blocking buffer (10 ml PBS, 2% pig serum, and 2% mouse serum). After this, rabbit anti-hLf was added for 60 min. Sections were washed, and the secondary biotinylated Ab (anti-rabbit IgG; Vector Laboratories, Burlingame, CA) was added for 30 min. Peroxidase activity was blocked by incubating the sections for 30 min in 0.3% H2O2 in methanol. Sections were incubated with Vectastain Elite ABC reagent (Vector Laboratories), washed in PBS, and incubated in peroxidase substrate solution until the desired stain intensity developed. Finally, sections were rinsed in tap water, counterstained with hematoxylin, cleared, and mounted in DPX.

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FIGURE 1. IFN-γ plasma levels were similar in hLf-transgenic mice (●) and wild-type (■) mice. All data are the mean ± SD of triplicate culture pooled from three mice and are representative of two experiments in which similar data were obtained.
Spleen cell proliferation and cytokine production. Because serum cytokine levels may reflect several independent factors, including rate of synthesis, tissue uptake, renal clearance, and presence of soluble inhibitors (e.g., receptors), all of which will be variable during active infection, we next sought in vitro evidence for altered immune responses in transgenic mice. Spleen cells from hLf-transgenic mice harvested 2–3 days after infection displayed significantly increased proliferative responses in vitro upon stimulation with either TSST-1, an exotoxin secreted by the S. aureus LS-1 (Fig. 2a), or heat-killed S. aureus LS-1 (Fig. 2b). Significantly enhanced proliferation to the SEA was also observed in transgenic mice after 2 days of infection (data not shown). In contrast, on day 0 the response to TSST-1 was significantly greater in the wild-type mice, suggesting that a priori the presence of hLf is immunomodulatory.

Spleen cells from hLf-transgenic mice cultured with TSST-1 in vitro generated significantly higher levels of TNF-α (Fig. 3) than did spleen cells from wild-type mice throughout the course of the experiment. IFN-γ production was also significantly higher in cultures from hLf-transgenic mice than in wild-type controls on days 3 and 7 after infection (Fig. 4). When cells were stimulated with SEA, significantly enhanced IFN-γ production was also observed in hLf-transgenic mice (data not shown). Because these data suggested type 1 T cell polarization, we next investigated the expression of cytokines typical of type 2 responses. In contrast, IL-5 production was significantly higher upon TSST-1 stimulation in cultures from wild-type mice on day 1 after infection (Fig. 5a), and IL-10 production was higher in cells from wild-type mice on day 3 (Fig. 5b; IL-4 levels were too low to be reliably assayed). These observations are consistent with the presence of a dominant type 1...
T cell response during staphylococcal infection, which is further enhanced in hLf-transgenic mice.

Immune responses in uninfected mice. Because it was observed that proliferation of spleen cells stimulated with TSST-1 on day 0 (i.e., uninfected mice) was greater in wild-type than hLf-transgenic mice (cf, Fig. 2a; the opposite of the responses in infected mice), the immune responses in uninfected mice were studied in more detail. Proliferation in response to TSST-1 was consistently greater in wild-type mice than in hLf transgenics at all concentrations of TSST-1 (Fig. 6a). Moreover, IFN-γ production in response to TSST-1 was consistently greater in spleen cell supernatants of wild-type mice (Fig. 6b), and there was also a significant difference, albeit less marked, in TNF-α production (Fig. 6c). Levels of IL-5 and IL-10 were too low for reliable quantification. Thus, the enhanced type 1 T cell response observed during infection in hLf-transgenic mice does not simply reflect a priori type 1 polarization of responses to bacterially derived superantigen.

Clinical effect of S. aureus infection in Lf-transgenic and control mice

hLf-transgenic mice cleared the bacteria more readily than wild-type mice (Table I); 28% of the transgenics were free of bacteria in both kidney and joints (the only tissues in which significant numbers of bacteria were found) at the end of the experiment, compared with only 7% of wild-type mice ($p = 0.027$, using $\chi^2$ test). There was a trend to higher mortality evident in wild-type (30%) compared with hLf-transgenic (21%) mice, although this was not significant. Clinically detectable infectious arthritis at 10 days postinoculation occurred in more wild-type than transgenic mice (27 vs 10%), although, as with mortality, this difference did not reach significance ($p = 0.12$, using $\chi^2$ test). The overall incidence of arthritis in these C57BL background mice was much lower than that in Swiss mice infected at the same time using the same protocol and bacterial load (data not shown). Uninfected mice, both transgenics and wild type, remained normal throughout.

Collagen-induced arthritis

The above observations suggested that mice carrying the hLf transgene mounted a more pronounced Th1 response following S. aureus infection compared with controls, but that the effect on clinical outcome was less clear-cut, with only bacterial elimination...
achieving statistical significance. Resistance to *S. aureus* depends on both antimicrobial and anti-inflammatory activities; a Th1 response is beneficial for the former, but could exacerbate the latter, which might explain the lack of a clear protective effect of the hLf transgene.

To investigate this possibility, the effect of the hLf transgene on the development of CIA was investigated. This Th1-driven arthritis lacks an infectious component but reflects an autoimmune response to collagen. It was found that the development of arthritis was indeed significantly more severe in the transgenic mice (Fig. 7; \( p < 0.05 \)).

**Antimicrobial activity of Lf**

The above results clearly indicate that the systemic presence of hLf has differing effects on *S. aureus* infection and CIA. A likely explanation for the superior microbial clearance in the transgenic mice during *S. aureus* infection is that hLf can directly inhibit microbial growth. Therefore, the ability of hLf to inhibit in vitro growth of *S. aureus* was tested. It was found that hLf reduced the growth of *S. aureus* LS1 in a low iron CDM, and this effect was reversible by iron (Fig. 8).

**Iron metabolism in hLf-transgenic mice**

There is some evidence that iron availability can affect the polarization of T cell responses, including autoimmunity (22). Because Lf is an iron-binding protein and can modulate iron supply to T cells (6) as well as to bacteria, we investigated whether the altered immunoregulatory function in hLf-transgenic mice was reflected by changes in their iron metabolism. It has been previously reported that serum levels of Lf are elevated in infection (23). Transgenic mice expressed high levels of hLf in plasma (Table II), but no significant change in plasma hLf levels was found on any day after infection. In contrast, plasma levels of endogenous mouse Lf did indeed increase in both hLf-transgenic and wild-type mice following infection. This presumably reflects the fact that hLf is constitutively synthesized by various tissues in the transgenic mouse and is not influenced by neutrophil degranulation, as would be the case for the endogenous mouse Lf.

Levels of non-heme iron in spleen and liver were similar in both groups of mice and did not alter during the course of infection. Plasma iron levels increased slightly during infection, but there was no difference between the groups, and hemoglobin levels and liver iron were also identical (data not shown). However, when spleen sections from noninfected hLf-transgenic mice and wild-type controls were stained for iron with Perl's Prussian Blue reaction (Fig. 9, \( a \) and \( b \)) iron deposits were observed in spleens from hLf-transgenic mice (Fig. 9\( a \)), but there was hardly any staining in wild-type spleens (Fig. 9\( b \)). Iron accumulated mainly in the red pulp, possibly in macrophages. This staining correlated with the distribution of hLf in the spleens of the transgenic mice (Fig. 9\( c \)), while there was no staining for hLf in the spleen of wild-type mice, as expected (Fig. 9\( d \)). However, after 3 days of infection iron deposits were seen in the spleen of both transgenic and wild-type mice (Fig. 9, \( e \) and \( f \)), and the distribution resembled that seen in the uninfected transgenic mice.

**Discussion**

The nature of the T cell response to infectious agents or inflammatory stimuli can have a major effect on the outcome of the disease. Th1 responses leading to macrophage activation are protective during most intracellular infections, but can exacerbate noninfectious states, including autoimmune diseases (24). Many factors can influence the polarization of T cell responses, including local iron availability. The aim of this work was to investigate a potential role for the iron-binding protein Lf.

We have previously shown that local administration of Lf into the inflamed joints of mice with collagen-induced experimental arthritis reduced inflammation (11). In these studies we did not detect systemic modulation of Ag-specific immune responses. Because Lf is an iron-binding protein that can interfere with iron uptake into T cells (6), and because low iron availability is reported to favor a Th1 response (22), Lf might be expected to exert immunomodulatory effects in vivo. In this work we have investigated the systemic effects of Lf on T cell responses to *S. aureus* infection using transgenic mice carrying a functional hLf gene. The casematin promoter used in these mice results primarily in expression in the mammary gland but is leaky in some mouse lines (17, 25) (P. van Berkel and R. W. de Winter, unpublished observations), permitting ectopic expression in other tissues. This extramammary expression of Lf in the mouse line 2261 used here was confirmed in this study, in which significant concentrations of hLf (\( \sim 400 \) ng/ml) were found in the plasma of these mice, and hLf could be detected by immunocytochemistry in the spleen.

In *S. aureus*-infected mice it was found that spleen cells from transgenic mice proliferated more strongly in response to staphylococcal Ags either through conventional or superantigen recognition pathways (TSST-1, SEA, or heat-killed bacteria). The synthesis of IFN-\( \gamma \) and TNF-\( \alpha \) by these cells was also increased in transgenic mice, whereas IL-5 and IL-10 synthesis was higher in wild-type mice. This strongly suggests that hLf expression in the transgenic mouse enhances the Th1-type response that is normally associated with *S. aureus* infection (26).

![FIGURE 8.](http://www.jimmunol.org/) hLf inhibits the growth of *S. aureus* LS-1 in vitro. Bacteria were grown in a defined minimal medium (see text), either unsupplemented (●) or supplemented with native (i.e., largely iron-free) hLf (100 \( \mu \)g/ml; ▲) or with hLf plus 50 \( \mu \)M iron, added as iron-nitritotriacetate (▲). The experiment was performed three times; data are single readings from a representative experiment.

### Table II. Levels of plasma hLf and mouse lactoferrin (mlf) in hLf-transgenic mice and wild-type mice after *S. aureus* infection

<table>
<thead>
<tr>
<th>Days After Infection</th>
<th>hLf-Transgenic Mice (+/-)</th>
<th>Congenic (---) Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hLf</td>
<td>mLf</td>
</tr>
<tr>
<td>0</td>
<td>302 ± 104</td>
<td>106 ± 23</td>
</tr>
<tr>
<td>3</td>
<td>241 ± 100</td>
<td>258 ± 155</td>
</tr>
<tr>
<td>7</td>
<td>277 ± 68</td>
<td>251 ± 127</td>
</tr>
</tbody>
</table>

*a* Results are measured in nanograms per milliliter.  
*b* ND, Not detectable.
Because the Th1 response against *S. aureus* is protective (27), it seems likely that this enhancement is responsible for the improved bacterial clearance in the transgenic mice. However, there is a two-edged nature to immune response polarization in bacterial infection. T cell subsets bearing Vβ11 TCR have been clearly implicated in septic arthritis disease progression (28). Thus, protective Th1 responses may clear bacteria but simultaneously exacerbate inflammatory responses. Similarly, macrophages may be both antimicrobial and proinflammatory in the mouse *S. aureus* arthritis model (29). Commensurate with these observations, IFN-γ blockade reduces the severity of septic arthritis in Swiss mice, directly implicating host Th1 responses in clinically detectable murine arthritis, whereas treatment with IFN-γ decreases mortality but enhances the development of arthritis (30). This may explain why the incidence of arthritis did not differ significantly between transgenic and congenic controls in our studies. To further investigate this possibility, we backcrossed the Lf transgene to the DBA/1 strain that develops chronic arthritis associated with a Th1-polarized response to type II collagen (CIA). We found that CIA in Lf-transgenic DBA/1 mice was characterized by significantly increased severity of joint inflammation.

The ability of hLf to cause Th1 polarization did not reflect prior Th1-type predisposition of the transgenic mice. Whereas cells from infected transgenic mice showed an enhanced Th1 response to *S. aureus* Ags in vitro, the opposite effect was seen with cells from uninfected mice. Thus, TSST-1 and (at high concentrations) heat-killed *S. aureus* generated greater proliferation and type 1 cytokine production by wild-type than by hLf-transgenic spleen cells. TSST-1 is known to act as a
superantigen (31), and it may be that hLf has a dual action. In uninfected mice it has a general down-regulatory effect on T cell proliferation, as has been previously reported for human T cells (6, 7), but once an infection with an associated Th1 response is established, hLf favors a Th1 over a Th2 response. The precise mechanism underlying this phenomenon remains unclear, but it may reflect interference with the increased iron requirement of T cells following activation (32).

We have previously reported that Lf administered peripherally to mice with joint inflammation, in both CIA or septic arthritis, reduced the degree of joint inflammation (11). This is in contrast to and apparently contradicts the increased joint inflammation evident in hLf-transgenic mice with CIA. The mode of delivery of Lf appears crucial to its function. Local administration of hLf to animals with established joint inflammation is unlikely at that stage to influence the systemic immune response. We have proposed that the mode of action of locally administered Lf is via sequestration of potentially toxic free iron in the inflamed joint, a mechanism that would operate equally well in both septic arthritis and CIA (20). In addition, Lf probably has a direct antibacterial effect on S. aureus. It has long been known that Lf inhibits the growth of many bacteria, including S. aureus, by virtue of its ability to sequester iron and render it unavailable to microorganisms (1), and indeed such an effect was found in vitro with the LS-1 strain of S. aureus used in this work (Fig. 8). The hLf-transgenic mice showed an enhanced ability to clear S. aureus compared with wild-type littermates, and others have shown that iv administration of hLf protected mice against experimental kidney infection (33).

Sequestration of iron would be expected to exert quite distinct effects on systemic immune responses to the foregoing local activities. To assess whether the altered T cell responses in the hLf-transgenic mice were related to alterations in iron metabolism, we examined the iron status of these mice. No differences were found between transgenics and control mice with regard to plasma and liver iron or hemoglobin levels. The same was true with mice infected with S. aureus. This is perhaps not surprising, as Lf plays no role in the main pathways of mammalian iron metabolism, we examined the iron status of these mice. No differences were found between transgenics and control mice with regard to plasma and liver iron or hemoglobin levels. The same was true with mice infected with S. aureus. This is perhaps not surprising, as Lf plays no role in the main pathways of mammalian iron metabolism, and it has recently been reported that Lf-knockout mice also display a normal iron phenotype (34). However, although total spleen iron measured chemically was similar in transgenic and wild-type mice, Perls-positive iron deposits were detected in the spleens of transgenic mice, but not in wild-type controls, and these colocalized with staining for hLf. This was observed in both infected and uninfected mice, and serum levels of hLf did not change upon infection of the transgenic animals. However, in wild-type mice in which splenic iron deposits were initially absent, Perls-positive staining appeared following infection, correlating with an increase in plasma levels of the endogenous mouse Lf. It is possible that accumulation of Lf in the spleen alters the relative distribution of iron within this organ, perhaps by removing it from the extravascular environment and depositing it in spleen cells such as macrophages, where it forms Perls-positive deposits. This could affect the initial immune response of spleen cells. However, the antimicrobial and immunomodulatory activities of Lf have also been ascribed to an iron-independent mechanism mediated through the basic N-terminal region of the Lf molecule (3), and the participation of such a mechanism in the present report cannot be ruled out.

Why should hLf in mice affect T cell polarization and the response to S. aureus when endogenous murine Lf already present might be expected to carry out similar functions? Murine Lf differs significantly from the human protein. In particular, it lacks the sequences of basic N-terminal amino acids that give rise to the microbicidal and anti-inflammatory effects of this region in human and bovine Lf (35). Moreover, its affinity for iron is lower than that of the human and bovine proteins (36), suggesting that it may function less well as an iron-binding protein in inflammatory foci. The biological role of Lf in the mouse may therefore differ from that in man. However, that fact that mouse Lf levels increased following infection, whereas hLf levels remained constant means that mouse Lf may also affect the response to S. aureus.

In conclusion, we have demonstrated that mice expressing hLf show modulation of their immune responses, manifest by polarization toward a Th1 response during active infection or Ag challenge. Lf has been proposed as a therapeutic agent. Our results clearly have important implications for such potential therapeutic use of Lf as an anti-inflammatory, antimicrobial, or immunomodulatory agent and indicate the necessity for a cautious approach in the first instance.

References


